In vivo evaluation of sixteen plant extracts on mice inoculated with *Trypanosoma brucei gambiense*

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After examination of the drugs used by traditional practitioners in Côte d'Ivoire, nine formulas prescribed in the treatment of African human trypanosomiasis (AHT) were selected for investigation. These formulas made use of 40 plants, 16 of which were studied because of their properties, as described in the literature, and their frequent use by practitioners. The plant extracts were administered, after maceration or decoction, either orally or intraperitoneally to Swiss mice that had previously been inoculated with Trypanosoma brucei gambiense *(Tbg), strain MHOM/CI/81/Dal 083. The parasitaemia in each mouse was followed for three consecutive days and compared with that in control mice, which had been given either a saline solution (SS: negative control) or well-known drugs (melarsoprol, difluoromethylornithine, and pentamidine: positive control).*

Our investigations led to the following conclusions. (a) None of the plant extracts revealed trypanocidal or trypanostatic activity relative to SS controls ($P > 0.05$). In fact, the mice that received the extracts died on the third day after inoculation, with 0% survival and an average parasitaemia of $10.8 \pm 2 \times 10^7$ trypanosomes/ml. (b) The treated positive controls, relative to SS, showed 100% survival and no parasitaemia ($P < 0.05$). Melarsoprol appeared to be active when given orally at a dose of 3.6 mg/kg body weight twice a day for 3 days.

This method of testing the sensitivity of trypanosomes to plant extracts is easy and inexpensive, and could be applied to other areas of research on tropical diseases.

Introduction

There are two distinct presentations of African human trypanosomiasis (AHT) depending on the type of *Trypanosoma brucei* (Tb) causing the disease: *T.b. gambiense* (Tbg, occurring in West Africa) and *T.b. rhodesiense* (Tbr, in East Africa). Some 50 million people are at risk in 36 endemic countries, with about 200 foci and 20000 reported new cases each year (*1*, *2*). In Côte d'Ivoire, since 1975, AHT has been an important public health problem. After a peak from 1977 to 1981 with about 500 cases each year, the number of patients decreased to 250 cases until 1986 as a result of control operations undertaken by the AHT Group of WHO in the Muraz Centre, Bobodioulasso, Burkina Faso, and selected rural hospitals.[a] At present, however, owing to the economic crisis worldwide, the number of cases is increasing again. For instance, the number of patients discovered in one study went up from 96 in 1990 to 155 in 1992.[a]

Melarsoprol, the most active drug currently used in the treatment of the second stage of AHT, is now facing problems of parasite resistance, relapses and side-effects (*2*).[b,c] Phytotherapy is therefore being resorted to by patients in developing countries owing to the low price and easy accessibility. Based on research (by *BBCY* for a degree in pharmacy),

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[a] **Miezan TB.** *Situation actuelle de la trypanosomiase humaine africaine en, Côte d'Ivoire.* PRCT, Daloa, Côte d'Ivoire, 1992 (unpublished document).

[b] **Assemien PAH.** *Les aspects épidémiologiques cliniques et thérapeutiques de la trypanosomiase humaine africaine, à Abidjan.* Thèse de medecine d'Abidjan. Université Nationale de Côte d'Ivoire, Abidjan, 1989.

[c] **Lathro A.** *Etat actuel de la thèrapeutique de la trypanosomiase humaine au stade neurologique.* Thèse de medecine d'Abidjan. Université Nationale de Côte d'Ivoire, Abidjan, 1985.

Table 1: **List of preparations from 16 plants for *in vivo* investigation**

No. (X)	Name of plant (Family)	Extracted from:	Drug prepared by: Maceration (MX)	Decoction (DX)
1.	*Annona senegalensis* (Annonaceae)	Dry leaves	M1	D1
2.	*Anogeissus leiocarpus* (Combretaceae)	Dry leaves	M2	D2
3.	*Bridelia ferruginea* (Euphorbiacea)	Dry bark of trunk	M3	D3
4.	*Cassia sieberiana* (Caesalpiniaceae)	Dry roots	M4	D4
5.	*Detarium microcarpum* (Caesalpiniaceae)	Fresh roots	M5	D5
6.	*Fagara zanthoxyloides* (Rutaceae)	Dry roots	M6	D6
7.	*Ficus thonningii* (Moraceae)	Dry roots	M7	D7
8.	*Gardenia aqualla* (Rubiaceae)	Dry roots	M8	D8
9.	*Guiera senegalensis* (Combretaceae)	Dry leaves	M9	D9
10.	*Khaya senegalensis* (Meliaceae)	Dry bark of trunk	M10	D10
11.	*Nauclea latifolia* (Rubiaceae)	Dry roots	M11	D11
12.	*Opilia celtidifolia* (Opiliaceae)	Fresh leaves	M12	D12
13.	*Parkia biglobosa* (Mimosaceae)	Dry bark of trunk	M13	D13
14.	*Piliostigma thonningii* (Ceasalpiniaceae)	Dry bark of trunk	M14	D14
15.	*Rauvolfia vomitoria* (Apocynaceae)	Dry roots	M15	D15
16.	*Securidaca longepedunculata* (Polygalaceae)	Fresh roots	M16	D16

the present study investigates the *in-vivo* activity of extracts from 16 plants, which are often prescribed in the treatment of AHT by traditional practitioners, so that we could identify the most effective plants for further research.

Materials and methods

As previously described,[d] nine formulas composed of 40 plants, which are used frequently in the treatment of AHT by traditional practitioners, were identified in the literature (*3*, *4*),[e] and 16 of them were selected for the present study.

The plants were identified by Professor Ake-Assi (Head, National Centre of Botany, Abidjan, Côte d'Ivoire) and prepared, based on directions given by the traditional practitioners, for maceration or decoction in the Department of Pharmaceutical Sciences of Abidjan University, as previously described.[d] The 16 drugs are listed in Table 1, where MX represents maceration and DX a decoction of substance X (X = 1 to 16 for each of the 16 plants).

[d] **Youan BBC.** *Evaluation biopharmaceutique de l'activité trypanocide de 41 extraits de plantes proposés en pharmacopée traditionnelle en Côte d'Ivoire.* Thèse de pharmacie d'Abidjan, Université Nationale de Côte d'Ivoire, Abidjan, 1993.

[e] **Ake-Assi YA.** *Contribution au recensement des espèces végétales utilisées traditionnellement sur le plan zootechnique et vétérinaire en Afrique de l'Ouest.* Thèse de diplôme d'état de medecine vétérinaire. Lyon, 1992.

Immediately after preparation, the samples were frozen at −20 °C in brown sealed bottles and then dispatched, in appropriate conditions, for *in-vivo* evaluation by PRCT (Project of Clinical Research on Trypanosomiasis) in Daloa.

In vivo *evaluation*

The strain of trypanosome we used had been isolated from the cerebrospinal fluid of a patient from Zuenoula (Côte d'Ivoire) in February 1981 by the method described by Cattan et al. (*5*). This strain increased in strength after 502 passages in mice and has been kept frozen in liquid nitrogen at PRCT, Daloa, since 1982. It was named Tbg strain MHOM/CI/81/Dal 083.

A total of 400 Swiss mice (aged 4–6 weeks; average weight, 23 g) were provided by PRCT, Daloa, and the Pasteur Institute, Côte d'Ivoire, and divided in groups of 4 by cage. The plant extracts were administered orally (0.30 ml per 10 g body weight) twice a day for 3 days, as proposed by the traditional practitioners, or by a single intraperitoneal injection (0.10 ml of extract, rendered isotonic with NaCl, per 10 g body weight). The mice were first given a single inoculation of Tbg strain MHOM/CI/81/Dal 083; 24 hours later, the plant extract or control drug was administered and the parasitaemia was followed daily for 4 days using a light microscope (magnification ×40). The level of parasitaemia on the third day and the rate of mice survival were used for comparing the results between the test group of mice that received the plant extracts, the negative controls (given NaCl 0.9%,

Table 2: **Percentage of mice surviving after treatment with plant extracts (M1 to 16 and D1 to 16) or control substances.** SS = salt solution, MP = melarsoprol, PT = pentamidine, DFMO = difluoromethylornithine.

	% of mice surviving on fourth day	
	Oral	Intraperitoneal
SS	0% (0.3 ml/10g)[a]	0% (0.1 ml/10 g)[a]
MP	100% (3.6 mg/kg)	100% (3.6 mg/kg)
PT	100% (4 mg/kg)	100% (4 mg/kg)
DFMO	0% (300 mg/kg)	0% (100 mg/kg)
DFMO	100% (600 mg/kg)	0% (100 mg/kg)
M1 to 16	0% (0.3 ml/10 g)	0% (0.1 ml/10 g)
D1 to 16	0% (0.3 ml/10 g)	0% (0.1 ml/10 g)

[a] Figures in parentheses are the doses of administration by single peritoneal injection or orally (twice a day for three days).

SS), and the positive controls (given melarsoprol, MP; pentamidine, PT; or difluoromethylornithine, DFMO), as described elsewere.[d] The doses are summarized in Table 2.

The statistical analysis was based on Student's *t*-test transformed in *P*-values ($P > 0.05$ is not significant; $P < 0.05$ is significant).

Results

The results are summarized below and in Table 2.

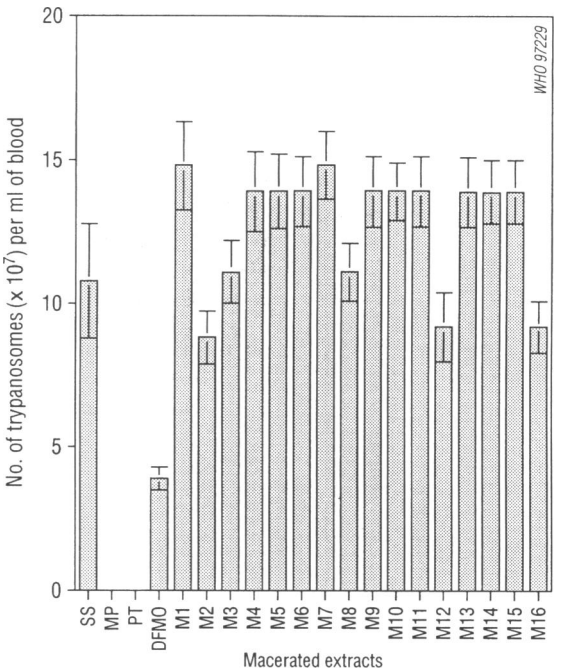
- *Effect of macerated extracts, given orally* (Fig. 1)

All four control mice treated with saline solution (SS) died on the third day (0% survival), with a parasitaemia of $10.8 \pm 2 \times 10^7$ trypanosomes per ml. Melarsoprol (3.6 mg/kg, twice a day) and pentamidine (4 mg/kg, twice a day) exhibited significant activity, compared with SS, with respect to both parasitaemia, which decreased gradually and was 0 on the third day, and survival, which was 100% ($P < 0.05$). The activity of DFMO (300 mg/kg, twice a day) and of each macerated plant extract was not significantly different, compared with SS ($P > 0.05$).

- *Effect of decoctions, given orally* (Fig. 2)

The results for melarsoprol and pentamidine were similar to those mentioned above. When the dose of DFMO was doubled (600 mg/kg, twice a day), the results were significantly different, compared with SS ($P < 0.05$). None of the extracts by decoction showed any detectable activity ($P > 0.05$).

Fig. 1. **Level of parasitaemia on the third day after oral administration of 16 plant extracts (by maceration, M1 to M16), compared with controls.** SS = salt solution, MP = melarsoprol, PT = pentamidine, DFMO = difluoromethylornithine.



- *Effect of decoctions, given intraperitoneally* (Fig. 3)

A single intraperitoneal injection of melarsoprol (3.6 mg/kg) and pentamidine (4 mg/kg) showed significant activity, compared with SS, with respect to both parasitaemia, which decreased gradually and was 0 on the third day, and survival, which was 100% ($P < 0.05$). The activity of DFMO (100 mg/kg) and that of each extract by decoction was not significantly different ($P > 0.05$), compared with SS.

- *Effect of macerated extracts, given intraperitoneally* (Fig. 4)

The results of all the macerated plant extracts were similar to those obtained with the decoctions given intraperitoneally.

Fig. 2. **Level of parasitaemia on the third day after oral administration of 16 plant extracts (by decoction, D1 to D16), compared with controls.** SS = salt solution, MP = melarsoprol, PT = pentamidine, DFMO = difluoromethylornithine.

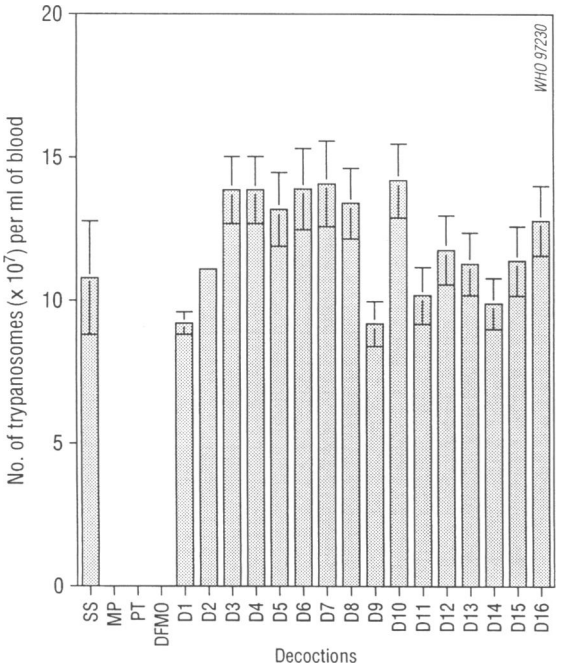
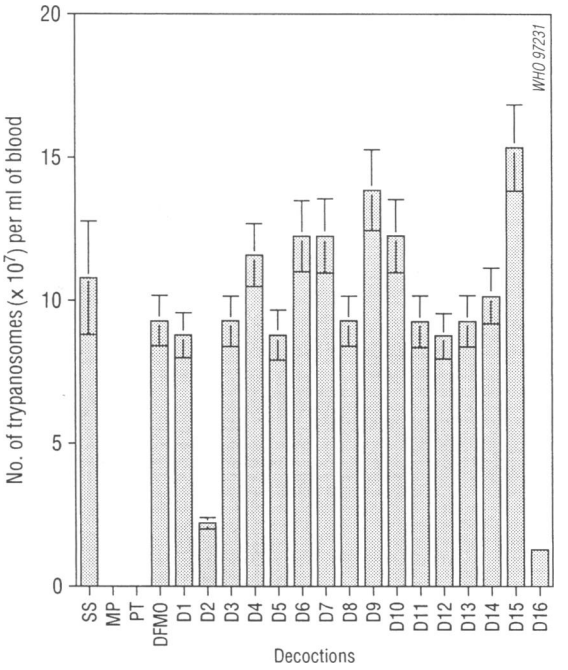


Fig. 3. **Level of parasitaemia on the third day after intraperitoneal injection of 16 plant extracts (by decoction, D1 to D16), compared with controls.** SS = salt solution, MP = melarsoprol, PT = pentamidine, DFMO = difluoromethylornithine.



Discussion

Oral administration

The idea behind the oral administration of these plant extracts to mice was that if the doses prescribed by the traditional practitioners are not toxic to their patients, they should not be toxic to mice, under the same conditions, and may even be active. The dose usually prescribed for the treatment of AHT is one glass of extract twice a day (approximately 250 ml/60 kg adult, twice a day). In mice, this is equivalent to 0.04 ml/10 g; but to obtain more rapid action, we gave 7.5-fold this dose (0.3 ml/10 g, twice a day). We found that none of the plant extracts, by maceration or decoction, was active in mice inoculated with Tbg strain MHOM/CI/81/Dal 083.

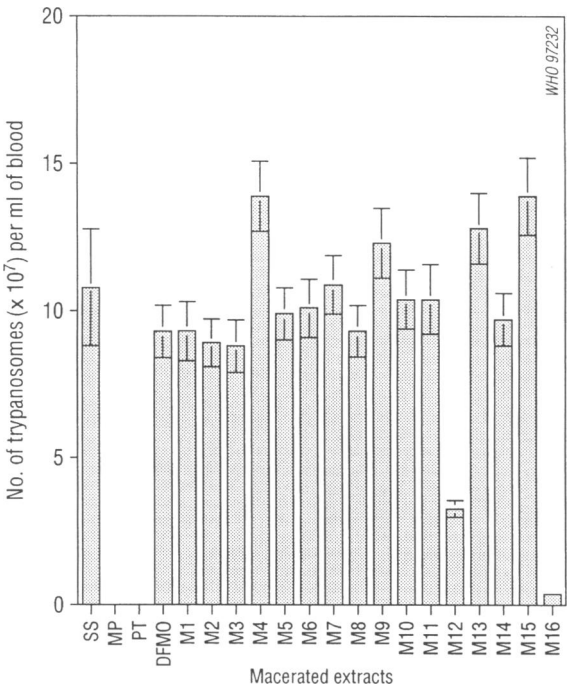
Considering the possibility of denaturation by heat during preparation of decoctions, we decided to test extracts of the plants by maceration to check whether any active ingredients were destroyed in the decoctions. Maceration was carried out at room temperature, while decoctions require heating to over 100 °C for about 1 hour. Our tests showed no difference in activity between the extracts by maceration and the decoctions. For all oral administrations, none of the extracts was toxic even with doses 7.5-fold higher than those prescribed by traditional practitioners; the mice died only because of the high level of parasitaemia.

DFMO was found to be active at a dose of 600 mg/kg, given orally twice a day for 3 days. In this situation, the DFMO seems to act before its complete elimination from the body.

Intraperitoneal administration

As no positive result was obtained by the oral route, intraperitoneal administration was undertaken in case some active ingredient could not be absorbed through the gastrointestinal system. All the plant extracts failed in our tests on infected mice. Sometimes, a toxic effect of the extract was noticed because of the low level of parasitaemia when death

Fig. 4. **Level of parasitaemia on the third day after intraperitoneal injection of 16 plant extracts (by maceration, M1 to M16), compared with controls.** SS = salt solution, MP = melarsoprol, PT = pentamidine, DFMO = difluoromethylornithine.



occurred, compared with the negative controls (SS). For example, D2 (decoction of *Anogeissus leiocarpus*) led to death of the mice on the second day, with a parasitaemia of $2.2 \pm 0.2 \times 10^7$ trypanosomes per ml, while M12 (macerated products of *Opilia celtidifolia*) induced death on the second day, with $3.3 \pm 0.3 \times 10^7$ trypanosomes per ml, and all our extracts of *Securidaca longepedunculata* (D16 and M16) led to death on the first day, with respective parasitaemias of 1.3 ± 0.13 and $0.4 \pm 0.04 \times 10^7$ trypanosomes per ml. The early death during low parasitaemia indicated that these four extracts (D2, M12, D16 and M16) had a toxic effect; when diluted with 0.9% sodium chloride, D2 (1:20), M12 (1:10), D16 (1:40) and M16 (1:50) were safe for animals but remained inactive. The lethal intraperitoneal dose for these extracts was therefore higher than these dilutions. We believe that toxicity of D2 might be due to its abundant tannins (*5*), while the saponin content might explain the toxicity of M12, D16 and M16.

DFMO was not active intraperitoneally at a dose of 100 mg/kg in mice; some trypanostatic activity was noticeable after 12 h but its systemic half-life was only about 3.5 hours (*6*, *7*).[f] In these conditions, a single intraperitoneal injection of DFMO is not sufficient. This fact justifies the administration by continuous perfusion in order to maintain a constant active blood concentration. For patients, compliance with long-acting DFMO delivery systems is needed.

The present study has allowed us to identify some of the plants prescribed in the treatment of AHT in Côte d'Ivoire, but our tests showed that none of their extracts was active in mice that had been inoculated with Tbg strain MHOM/CI/81/Dal 083. Evaluation of their activity in sheep showing symptoms of AHT would be an interesting area of research. This method of testing the sensitivity of trypanosomes to plant extracts is easy and inexpensive, and could be applied to other areas of research on tropical diseases.

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Résumé

Evaluation *in vivo* de 16 extraits de plantes chez des souris infectées par *Trypanosoma brucei gambiense*

Une prospection auprès des tradipraticiens a permis de recenser 9 formules proposées dans le traitement de la trypanosomiase humaine africaine. Ces formules utilisaient 40 plantes, dont 16 ont été retenues pour cette étude au vu des données de la littérature et de la fréquence de leur prescription. Ces plantes ont été administrées par voie orale ou intrapéritonéale, sous forme de macération ou de décoction, à des souris Swiss préalablement infectées par la souche MHOM/CI/81/Dal 083 de *Trypanosoma brucei gambiense*. La parasitémie

[f] See footnote *c*, page 343.

a été suivie chez ces souris pendant 3 jours consécutifs, par comparaison avec des témoins négatifs (souris traitées par du soluté physiologique seul) et des témoins positifs (souris traitées par des médicaments connus — mélarsoprol, DFMO et pentamidine).

Ces différentes investigations ont abouti aux conclusions suivantes: *a)* aucun des extraits testés ne présente d'activité trypanocide ou trypanostatique par rapport au soluté physiologique ($p > 0,05$). La survie des souris traitées était de 0% le troisième jour après l'inoculation, avec une parasitémie moyenne de $10,8 \pm 2 \times 10^7$ trypanosomes/ml; *b)* l'activité du traitement des témoins positifs a été confirmée, aux posologies habituelles, par rapport au soluté physiologique, avec une survie de 100% et une parasitémie finale nulle ($p < 0,05$). Le mélarsoprol s'est par ailleurs révélé actif par voie orale à la dose de 3,6 mg/kg 2 fois par jour pendant 3 jours; *c)* ce protocole nouveau et peu onéreux d'évaluation de la sensibilité des trypanosomes aux extraits de plantes ouvre de nouvelles voies de recherche sur les maladies tropicales (notamment dans le domaine de la phytothérapie).

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